Separation and Characterization of Cells with Varying Magnetic Nanoparticle Concentration

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Background:

Magnetic iron oxide nanoparticles (MNP) have the potential for use as a multimodal cancer therapy agent due to their ability to carry and release anticancer drugs, potential as radiation sensitizing agents, as well as creating localized heat when exposed to an alternating magnetic field (AMF). [1, 2] The size and coating on the MNP, as well as the method of delivery, affects the cellular uptake of MNP.[3] The mechanism for the uptake and distribution within cells is not fully understood. Optimization of targeted cellular uptake is critical if magnetically induced hyperthermia is to continued to be developed as a viable clinical cancer therapy.

Goal:

We propose to develop a method to separate cells based on intracellular MNP concentration which will then allow us to further study and optimize cellular uptake in tumor cells.

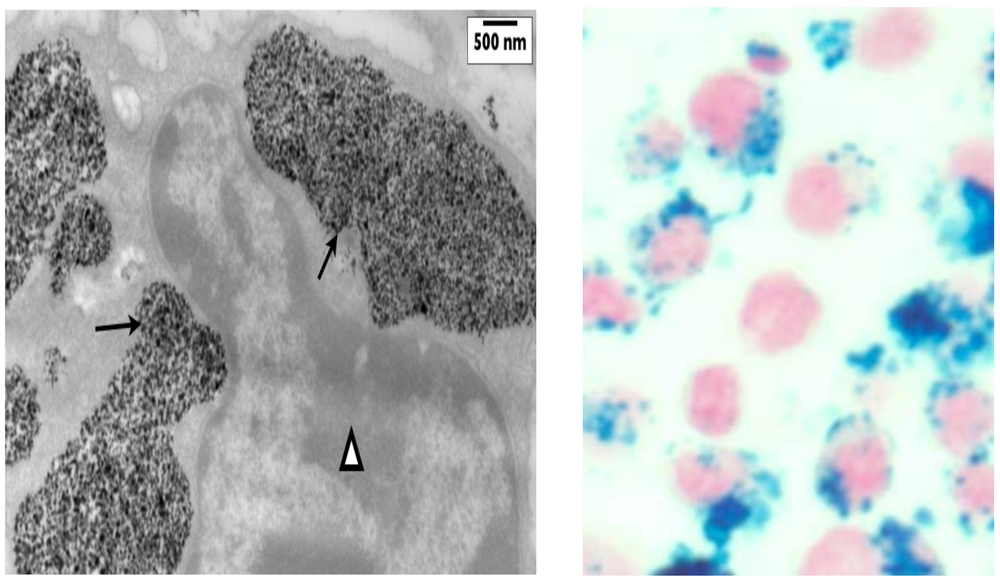

Figure 1: Left: Transmission electron microscopy (TEM) of tumor cell uptake of MNP. This TEM image MNP inside of vesicles within a murine adenocarcinoma cell (arrows). The cell's nucleus (triangle) is shown adjacent to the nanoparticle collections.[3] Right: MTGB cells incubated with MNP for 48 hours (*in vitro*). Prussian Blue stain indicates MNP (iron). Significant variation in MNP concentration appears to exist within the cell population.

Significance:

Current methods of MNP quantification consider the average iron concentration in a bulk sample (millions of cells), or are qualitative/2D methods which do not provide absolute iron values. By developing a method which separates living cells, we will be able better examine the mechanisms responsible for intracellular uptake. In addition, we will be able to quantify the variation of MNP concentration cell to cell which histology suggests exists.

Basic experimental outline for future experiments using proposed separation method:

We propose to use a solenoid which will create a magnetic field when current passes through the solenoid. The magnetic field created will be strong enough to separate the cells based on their intracellular MNP concentration. The following formula can be used to calculate the magnetic field.

$$B=(\mu NI)/ L$$

Where B is the magnetic field strength, N is the number of turns in the solenoid, I is the current and L is the length of the solenoid.

Cells will be cultured under optimum conditions and after 80% confluency is reached, the cells will be incubated with MNPs for 48 hours.

Methods of validation:

Transmission electron microscope (TEM): Characterization of particle uptake and endosome size.

Inductively coupled plasma mass spectroscopy(ICP-MS): Quantification of the iron content present in the cells (average value).

Histopathological analysis: Prussian blue staining to identify relative iron content in cells.

Variables to be explored in future work:

We intend to study the cell fractions obtained after the separation and study the gene expression profile of the key genes, the role of hypoxia during MNP uptake by cells, cell cycle analysis of the fractions and the metabolic profile of the cells. Variation in particle material and size will also be considered.

References:

1."Multifunctional superparamagnetic iron oxide nanoparticles for combined chemotherapy and hyperthermia cancer treatment." *Nanoscale*. U.S. National Library of Medicine, n.d. Web. 27 Feb. 2017.

2.Petryk, Alicia Ailie. *Magnetic nanoparticle hyperthermia as an adjuvant cancer therapy with chemotherapy*. Thesis. 2013. Dartmouth College, Hanover, NH, Print.

3. Giustini, Andrew. *Magnetic nanoparticle surface coating and tumor microenvironment alterations for improved cancer treatment efficacy*. Thesis. 2012. Dartmouth College, Hanover, NH, Print.